

# 2 Alternative Carbohydrate Reserves Used in the Daily Cycle of Crassulacean Acid Metabolism

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## 2.1 Introduction

Each day a massive interlocked biochemical cycle occurs in the green tissues of crassulacean acid metabolism plants. The function of this interlocked cycle, in its simplest context, is to furnish most of the  $\text{CO}_2$  for CAM plant photosynthesis. In addition, this diel (24 h) cycle produces the primary identifying marks of a CAM tissue through two ancillary cycles. One cycle involves a nocturnal acidification and its loss the next day, while the second concerns the depletion of a carbohydrate reserve at night and its replenishment the next day. Formally Benjamin Heyne (1815) is credited with writing, nearly two centuries ago, about the "acid as sorrel" taste of a succulent green plant at dawn and the "bland taste" caused by acidity loss later in the day. In fact, the exact origins of these observations are lost in antiquity, but certainly are referred to in Roman and Biblical writings. The circumstantial cause of the acidity was postulated to be an organic acid about a century ago and the bland taste later was associated with starch; but these ideas were not plainly coupled together in theory nor quantitatively studied until the late 1940s. Then, with the discovery of major portions of intermediary metabolism and the advent of additional quantitative biochemical procedures, the nature of the daily reciprocal relation between the acid and the bland taste was recognized and measured quantitatively. The acid taste is caused principally by malic acid, while the bland taste is caused by deacidification plus the reciprocal synthesis of a bland tasting carbohydrate, e.g. a polysaccharide such as starch.

Other daily ancillary cycles, e.g.  $\text{CO}_2$  and  $\text{O}_2$  exchange, stomatal functions, an internal pool of  $\text{CO}_2$ , etc. also exist as integral parts of CAM (Kluge and Ting 1978; Ting and Gibbs 1982; Winter 1985). The focus of this work, however, is on the type of carbohydrate and how each is metabolized in certain CAM plants when it functions as the daily carbon reservoir to provide the phosphoenolpyruvate (PEP) for nocturnal  $\text{CO}_2$  fixation and organic acid synthesis. Today alternative pathways of intermediary carbohydrate metabolism in plants are known (Sung et al. 1988) and alternative carbohydrate reserves are recognized in specific CAM species (Black et al. 1982) which can be either a polysaccharide or a

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neutral hexose sugar. Unfortunately, these disparate bodies of knowledge have not been fully integrated with CAM. Therefore, in this unified presentation our aims are (1) to divide CAM plants into two metabolic groups, (2) to document the use of different carbohydrate reserves by each group, (3) to integrate the unique biochemical reactions within each group into characteristic sets of metabolic pathways, thereby depicting two metabolic sequences of carbon cycling in CAM, and (4) to compare the bioenergetics and other features of these two metabolic groups.

## 2.2 The Division of CAM Plants into Two Metabolic Groups

The division of CAM plants into distinct metabolic groups was proposed when two types of  $C_4$ -acid decarboxylases were found in CAM plants. Malic enzyme was first assayed in certain CAM plants in the mid 1950s, but it was not widely studied nor considered to act as a decarboxylase until the late 1960s, only several years after the 1965 discovery of  $C_4$  photosynthesis (Black 1973). Thus in 1973 when a second decarboxylase, PEP carboxykinase, was found to be very active in specific CAM species, it was clear that two large groups of CAM plants existed (Dittrich et al. 1973). The details of these separate metabolic conversions were not clear, but, over the intervening two decades, new information about carbon metabolism has been found in higher plants and distinct metabolic variations have been discovered in other CAM plants.

Table 2.1 is a condensed presentation of earlier work on the taxonomic distribution of some carbon metabolism enzymes among CAM plants. In the initial work classifying CAM plants into two groups, the activities of PEP carboxykinase and pyruvate,  $P_i$  dikinase were used as the basis of division (Dittrich et al. 1973). Pyruvate,  $P_i$  dikinase shows a taxonomic pattern complementary to PEP carboxykinase (Table 2.1). But some enzymes were active in all CAM plants, however, with elevated activity in one group. For example, both types of malic enzyme tended to be expressed more in plants without PEP carboxykinase, whereas the pyrophosphate-dependent phosphofructokinase (PFK) tended to be more active in plants containing a highly active PEP carboxykinase. Even with these limitations, data as in Table 2.1 gave a reasonable basis for the division.

## 2.3 The Use of Soluble Sugars Versus Polysaccharides as a Carbohydrate Reserve

Starch was identified in early CAM work as the likely source of carbon for the nocturnal synthesis of organic acids (Bennet-Clark 1933; Wolf 1937). For several decades, research on CAM tissues was dominated by efforts to understand the night  $CO_2$  fixation,  $O_2$  metabolism, the respiratory quotient, and other daily gas exchange traits (Kluge and Ting 1978; Edwards and Walker 1983) (see also Chap. 1). Some clever ideas were published to "explain" the unusual gas exchange

**Table 2.1.** Taxonomic distribution of enzymes amongst CAM plants separated into two groups based on PEP carboxykinase activity

Family (number of species)	PEPCK <sup>a</sup>	NAD-ME <sup>a</sup>	NADP-ME <sup>a</sup>	PPDK <sup>a</sup>	PP <sub>i</sub> -PFK <sup>a</sup>
$\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$					
<b>Active PEP carboxykinase</b>					
Asclepiadaceae(3)	173-440	10-90	2-44	nil <sup>b</sup>	-
Bromeliaceae(12)	193-999	16-140	6-91	nil	25-107 <sup>c</sup>
Euphorbiaceae(2)	625-830	167-232	-	nil	-
Asphodelaceae(2)	178-480	29-62	-	nil	-
Vitaceae(1)	597	106	-	-	-
<b>Little PEP carboxykinase</b>					
Agavaceae(3)	ND <sup>b</sup>	43-785	144	40-50	-
Aizoaceae(2)	ND	195	-	-	-
Asteraceae(2)	ND	50	-	80	-
Cactaceae(3)	ND	47-920	131	30-200	2 <sup>c</sup>
Crassulaceae(5)	ND	140-385	46-212	90-240	2-8 <sup>c</sup>
Orchidaceae(3)	ND	60-190	151-217	30-200	-
Dracaenaceae(2)	ND	50-127	120	70	-

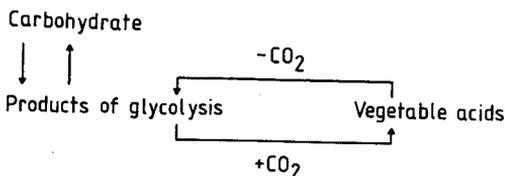
<sup>a</sup>PEPCK: PEP carboxykinase; NAD- or NADP-ME: malic enzyme; PPDK: Pyruvate, phosphate dikinase; PP<sub>i</sub>-PFK: pyrophosphate-dependent phosphofructokinase. The level of detection via the assays employed was  $< 5 \mu\text{mol mg}^{-1} \text{Chl h}^{-1}$ . Data collected about 1983 from Kluge and Osmond (1971); Dittrich et al. (1973); Sugiyama and Laetsch (1975); Black (1976); Dittrich (1976); Holtum and Osmond (1981); Black et al. (1982); and Carnal and Black (1983).

<sup>b</sup>Not detectable. A dash indicates no assay reported. Nil indicates a detectable activity but usually less than  $1 \mu\text{mol mg}^{-1} \text{Chl h}^{-1}$ .

<sup>c</sup>Activity in  $\mu\text{mol mg}^{-1} \text{protein h}^{-1}$ .

traits of whole CAM plant tissues. Nevertheless, there was little meaningful understanding of how CAM functioned biochemically.

Hence, while the general phenomenon of an acid taste in succulent plants was recognized for centuries, no integrated model existed until the late 1940s. Then in a remarkable effort to unify the current knowledge about CAM gas exchange, M. Thomas presented a scheme [initially in the third edition of his textbook (Thomas 1947) and subsequently in a research paper (Thomas 1949)] for the interconversion of the carbohydrate and "vegetable acids" as given below:



This simple loop-like scheme was given without further comment. Even so, in distilled essence, it is the model that guides the research on how CAM functions even today! These relationships were strongly supported in the series of quantitative

studies on total acids and carbohydrates by H.B. Vickery and coworkers (Pucher et al. 1949; Vickery 1954) who demonstrated that these diel synthesis and degradation processes were the reverse of each other, occurring in a reciprocal fashion each day.

These quantitative relationships between starch and malic acid quickly allowed them to fit their data into a similar scheme. For example with *Bryophyllum calycinum* (= *Kalanchoë pinnata*), starch loss and acid accumulation balanced (Pucher et al. 1949). Indeed, in *K. pinnata*, starch loss at night was 50% higher than required for acid synthesis (Sutton 1975a, b). Even though putatively the carbohydrate was starch, in some cases, starch could not account for the amount of acids synthesized. For example, in *K. tubiflora* and *K. daigremontiana*, starch accounted for only two-thirds of the carbon (Sutton 1975a, b), and in *Opuntia aurantiaca* less than 40% of the carbon was derived from starch (Whiting et al. 1979). In each of these plants however, the glucan (low-molecular-weight polymers of glucose) pool change was sufficient to account for the remaining carbon and these authors concluded that soluble sugar pools did not contribute carbon for PEP synthesis. But in other studies, on the effects of changing environments, Vickery (1954) could not totally account for the malate carbon from starch even in *B. calycinum* (*K. pinnata*), which underscores the strong responses of CAM to environmental conditions (Kluge and Ting 1978).

It is somewhat ironic that, simultaneously with this excellent work done in Connecticut on balancing acidity with a polysaccharide, the first work with soluble sugars as a potential carbon reserve appeared in the literature with pineapple from Hawaii (Sideris et al. 1948). The exact pathway of carbohydrate metabolism in pineapple leaves had not been understood because substantial amounts of sugars were depleted each night concurrent with malate synthesis. Indeed, about 7 to 8% of a pineapple leaf total dry weight was lost as soluble sugars at night. The irony is that these workers failed to appreciate that pineapple leaves conduct CAM! In other work soluble sugars also were reported to decrease concurrent with organic acid formation in *Nopalea cochinellifera*, with this decrease supplying about 15% of the carbon needed for CAM (Master 1959).

One additional aspect of CAM came from measuring the daily carbohydrate turnover. From that work it was possible to calculate that between 8 and 20% of the total dry matter in green CAM leaves was committed to the daily cycle (Vickery 1954; Black 1976; Black et al. 1982). This is a massive investment by a green tissue!

We have reviewed the literature for measurements of the stoichiometries between acidity and various types of carbohydrate reserves. These data can be quantitatively compared over a night period or a day period since they must at least balance; in fact, the carbohydrate reserve should be in excess to account for normal respiration, translocation, etc. Some of the older work was just cited and Table 2.2 presents some more recent data with CAM plants. It is fundamental to note that all green plant tissues contain both polysaccharides and hexoses. However, for CAM to function, the massive acid accumulation each night must be balanced by a substantial carbohydrate supply. Also note that, energetically,

Table 2.2. Changes in malate and carbohydrates from dusk to dawn for some CAM plants

Species <sup>a</sup>	Malate	Starch and glucans	Soluble sugars <sup>b</sup>	References
<i>Ananas comosus</i>	+84	-19	-19G, -20F, -3S	Carnal and Black (1989)
	+64, high PPF	15% of CH <sub>2</sub> O	-120	Borland and Griffiths (1989)
<i>Clusia minor</i> <sup>c</sup>	+90, low PPF	15% of CH <sub>2</sub> O	-54	Popp et al. (1987)
	+240 <sup>d</sup>	-31	-174G, -165F, +8S	
	+124 <sup>d</sup>	-	-92G, -79F, +5S	
<i>Clusia alata</i>	+63 <sup>d</sup>	-22	-45G, -38F, +10S	
	+83 <sup>d</sup>	-	-93G, -94F, -13S	
<i>Kalanchoë daigremontiana</i>	+225	-135	-5	Kenyon et al. (1981)
	+45	-60	-	Brunnhöfer et al. (1968)
<i>Sedum telephium</i>	+45, high <sup>e</sup> PPF	-37	-25	Borland and Griffiths (1992)
	+31, high <sup>e</sup> PPF	-40	-25	
	+10, high <sup>e</sup> PPF	-19	-21	
	+32, low <sup>e</sup> PPF	-6	-4	
	+11, low <sup>e</sup> PPF	-3	-11	
	+4, low <sup>e</sup> PPF	-2	-12	

<sup>a</sup>Data on other species are also available: Griffiths et al. (1989) on *Pyrrhosia piloselloides*; Medina et al. (1986) on *Bromelia humilis*; Ball et al. (1991) on *Clusia rosea*; and Paul et al. (1993) on *Mesembryanthemum crystallinum*.

<sup>b</sup>Soluble sugars are totals, or G = glucose, F = fructose, S = sucrose. All values are in  $\mu\text{mol}$  equivalents of hexoses or  $\mu\text{equivalents}$  assuming  $2\text{H}^+$  per malate per gramme fr. wt. of tissue.

<sup>c</sup>This species was originally described as *C. rosea* (Popp et al. 1987), but was subsequently shown to be *C. minor* (Franco et al. 1991).

<sup>d</sup>Collected at different seasons.

<sup>e</sup>Collected with tissues at different relative water contents.

carbohydrates cost much less to turn over than would a protein or a lipid, hence the latter are not considered likely carbon sources (see also Chap. 24). Vickery and others considered this idea earlier in CAM research and reached this conclusion also.

Again, from the data in Table 2.2, CAM plants can be divided into two groups based on balancing acidity either with a water-insoluble polysaccharide, e.g. starch or glucans, or with a water-soluble hexose. In some CAM plants the acid can be completely balanced by polysaccharides, e.g. *Kalanchoë daigremontiana*; but in *Ananas* and *Clusia* acidity is balanced by hexoses. In most measurements one or the other dominates (Table 2.2).

As a more detailed example, we have measured the stoichiometries of malate to various forms of carbohydrates in *Ananas* (pineapple) leaves (Black et al. 1982; Carnal and Black 1989, Table 2.2) that can form 84  $\mu\text{mol}$  of malate at night per gramme fresh weight. Approximately 19  $\mu\text{mol}$  (or 38  $\mu\text{mol}$  malate equivalents) of glucose, as starch plus glucans, were used at night. Thus, glucans are not sufficient as a carbohydrate source to furnish the carbon to form PEP for malate synthesis. However, the soluble sugar pool in pineapple leaves is large and about 42  $\mu\text{mol}$  of hexose equivalents were depleted overnight. The soluble sugar pools involved are glucose and fructose which, within pineapple leaf cells, are stored in the vacuole (Kenyon et al. 1985). Therefore, in these pineapple leaves counting polysaccharides and hexoses, a total of approximately 61  $\mu\text{mol}$  of glucose equivalents were metabolized at night. Since 42  $\mu\text{mol}$  of hexose equivalents would be needed to form 84  $\mu\text{mol}$  of malate, the leaf has 19 glucose equivalents remaining for other types of night carbohydrate metabolism, translocation, etc. Therefore, with pineapple leaves in CAM, the soluble sugar pool furnishes, as a minimum, half of the carbon for PEP synthesis at night and maximally could account for all of the PEP. The leaf sucrose shows little diurnal change in its pool size; but note that pool size is *not* a measure of sucrose turnover. Presumably, sucrose must turn over rapidly to support the rapid growth of pineapple.

Such detailed work has not been performed with other hexose-utilizing CAM tissues. Nevertheless, from the data in Table 2.2 we can classify *Ananas* and *Clusia* as hexose-utilizing CAM plants. Also, a short report appeared on *Aloë arborescens* showing that mannose and galactose can serve as carbohydrates to balance the daily acidity (Verbücheln and Steup 1984). Unfortunately, there has been no further work on this species. We conclude that glucose and fructose are hexoses that CAM plants can commonly use for PEP synthesis each night and that other hexoses such as mannose and galactose can be used if the *Aloë* results are confirmed and the required hexose metabolism is elucidated.

The results in Table 2.2 also underscore how much the daily amplitude of CAM can vary with seasons of the year and other changes in environment. The *Clusia* data were collected at two seasons; and light intensity and tissue water content show strong influences on pineapple and *Sedum* (Table 2.2). This environmental responsiveness of CAM plants has been known for decades. In controlled-environment studies on photoperiod and thermoperiod, it was shown that extremes such as continuous light or darkness, or constant temperature, can

depress the daily amplitude of CAM to zero in about 5 to 7 days. Conversely, CAM will recover in about the same time when the plants are given environmental parameters that have more normal daily fluctuations (Crews et al. 1976; Chang et al. 1981).

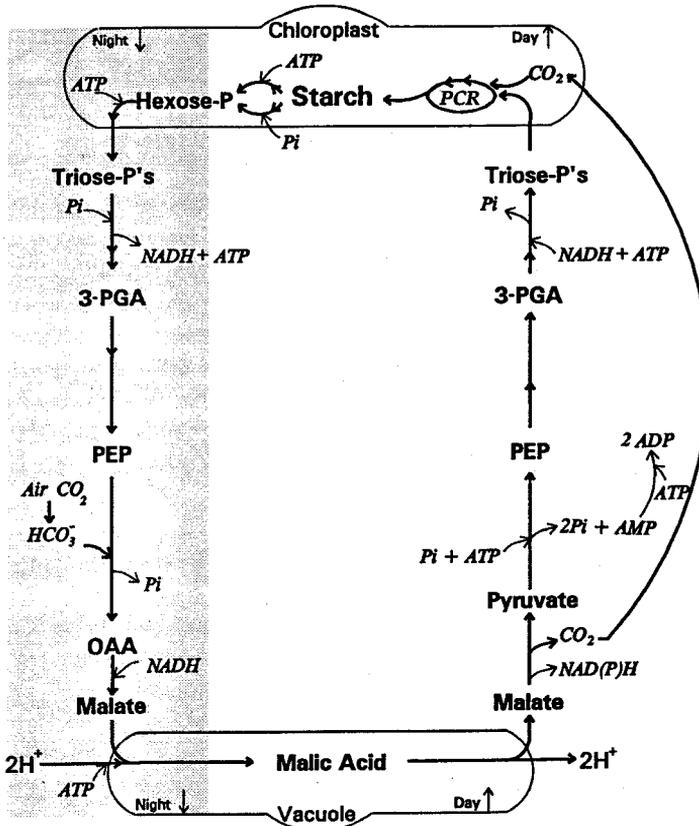
## 2.4 Sequences of Biochemical Reactions in the Daily Use of Hexoses Versus Starch in CAM

Accepting that a polysaccharide or a hexose can serve as the carbohydrate reserve in the diurnal cycle of CAM also leads to the understanding that the sequences of biochemical reactions will be different and the subcellular sites of carbohydrate storage will vary. Almost certainly, starch and glucans are synthesized and stored in the chloroplast. In contrast, free hexoses are most likely to be synthesized in the cytosol and stored in the vacuole. Therefore, a different sequence of biochemical reactions is required in each group of CAM plants. Collectively from these carbohydrate reserve studies (Table 2.2) we can realize that hexose-utilizing species also have a high activity of PEP carboxykinase, while the polysaccharide-utilizing species have malic enzymes as decarboxylases (Table 2.1).

In addition, new information has accrued in plants about intermediary carbohydrate metabolism that can be integrated into the pathways of carbon flow between, for example, pyruvate and sucrose. That information includes the use of  $PP_i$  as an energy source, the presence of several alternative routes to interconvert F 6-P and F 1,6- $P_2$ , and the widespread use of various nucleoside triphosphates (NTPs) by kinases within intermediary metabolism (Sung et al. 1988).

Other unique features of plant metabolism also must be factored into their pathways of metabolism. In animal cellular metabolism, it is common to dephosphorylate G 6-P in order to translocate glucose in the bloodstream. But there is little convincing evidence that plant cells can dephosphorylate either glucose or fructose monophosphates. Hence it is not common for plants to synthesize free glucose or fructose. Rather, in plants free sugars are hydrolyzed from polymers, e.g. starch, glucans, fructans, or sucrose and its derivatives. Indeed, in plants the most direct biochemical route to form both glucose and fructose is first to synthesize sucrose and then to hydrolyze it with invertase. Therefore, this new information and these peculiarities of plant metabolism must be integral portions of each daily CAM cycle.

Figures 2.1 and 2.2 are complete daily metabolic cycles for the CAM plants whose traits were illustrated in Tables 2.1 and 2.2. Figure 2.1 is for a polysaccharide (i.e. starch/glucan) cycle plant. This is the type of CAM cycle formulated essentially in parallel with similar discoveries about  $C_4$  and  $C_3$  photosynthesis in the 1960s and 1970s. For example, it includes: a  $C_4$ -acid decarboxylation by either an NAD- or NADP-dependent malic enzyme; the conversion of pyruvate to PEP by pyruvate,  $P_i$  dikinase; the utilization of NADH by the cytosolic glyceraldehyde 3-P dehydrogenase; and the transport of triose phosphates across the chloroplast envelope. Also the two accumulating metabolites, starch and



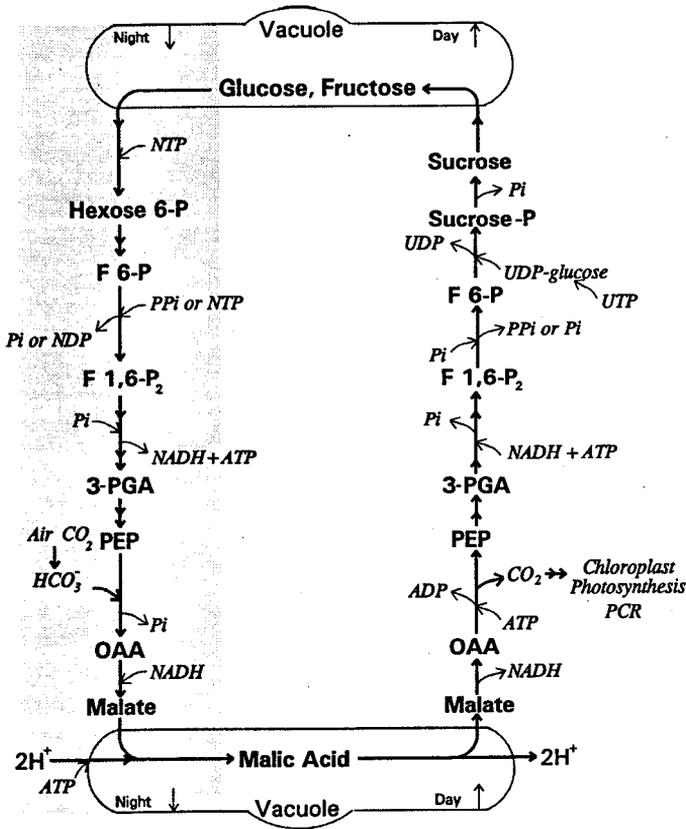
**Energetics:**



**Fig. 2.1.** Biochemical cycle for the daily flow of carbon in polysaccharide-cycling CAM plants. The major accumulating compounds, starch and malic acid, are temporally stored, day versus night, in the chloroplast and the vacuole. Starch breakdown in the chloroplast could be either amyolytic or phosphorolytic, but the overall energy budget for starch synthesis has been calculated assuming the latter. PCR Photosynthetic carbon reduction cycle

malic acid, are stored in separate organelles: the NAD-malic enzyme likely is in the mitochondrion, and most evidence shows the pyruvate, P<sub>i</sub> dikinase is in the chloroplast. Hence, within these green CAM cells, the entire daily cycle involves three organelles and the cytosol.

The cycle in Fig. 2.2 is a unified presentation that integrates current information about CAM plants that decarboxylate C<sub>4</sub> acids via PEP carboxykinase. This daily hexose cycle is for plants such as pineapple that utilize glucose and fructose as their carbohydrate reserve. We presented portions of this cycle earlier (Dittrich et al. 1973; Black et al. 1982; Carnal and Black 1989); but several newer features

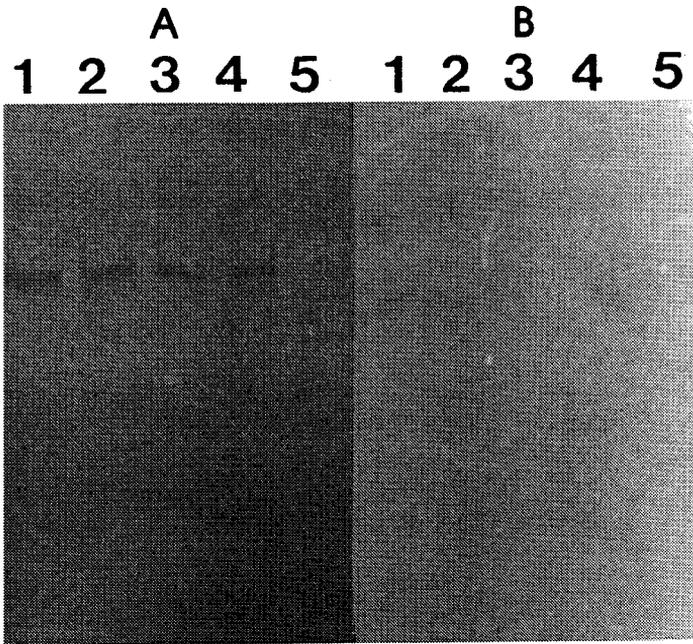


**Energetics:**



Fig. 2.2. Biochemical cycle for the daily flow of carbon in soluble-hexose-cycling CAM plants. The major accumulating compounds, hexoses and malic acid, are temporally stored, day versus night, in the same subcellular organelle, the vacuole. The overall energy budget has been calculated treating NTP and UTP as ATP equivalents

have been added. One is the use of alternative NTPs by hexokinases and phosphofruktokinase at night and the other is the synthesis of sucrose to produce the storage hexoses, glucose and fructose, each day. As already stated, plants seemingly lack hexose monophosphate phosphatases; therefore they cannot produce free hexoses readily, particularly in the near equal quantities of glucose and fructose as found in pineapple leaves (Table 2.2). Rather the most direct biochemical route for an equal hexose synthesis is through sucrose synthesis followed by its cleavage with invertases. We postulate that the soluble acid invertase hydrolyses sucrose in the pineapple cell vacuole (Fig.2.2). The daily storage of hexoses in pineapple vacuoles has been demonstrated (Kenyon et al. 1985).



**Fig. 2.3.** Western immunoblotting for invertase in CAM plants. Equal amounts (20  $\mu$ g) of soluble proteins from the following CAM plants were loaded in each lane of two SDS-PAGE gels. Both gels were run simultaneously, then they were transferred to nitrocellulose papers and immunoblotted with antibodies against soybean soluble acid invertase. *Left:* lane 1, *Billbergia amoena* (Bromeliaceae); lane 2, *Ananas comosus* (pineapple, Bromeliaceae); lane 3, *Hoya carnosa* (Asclepiadaceae); lane 4, *Aloë arborescens* (Asphodelaceae); lane 5, *Peperomia obtusifolia* (Piperaceae); *Right:* lane 1, *Kalanchoë daigremontiana* (Crassulaceae); lane 2, *Kalanchoë tubiflora* (Crassulaceae); lane 3, *Sedum telephium* (Crassulaceae); lane 4, *Pachyphytum oviferum* (Crassulaceae); lane 5, *Crassula lycopodioides* (Crassulaceae)

Therefore, in this group of CAM plants, the vacuole is the subcellular site for acid accumulation at night and for hexoses in the day, and the cytosol is the site of  $C_4$ -acid decarboxylation.

Recently, we also have studied some of the enzymatic requirements of both Figs. 2.1 and 2.2 in representative plants from these two CAM groups. Table 2.3 summarizes some of these results and shows that enzymes do tend to be expressed in accordance with both daily cycles in specific plants. While the enzyme data in Table 2.3 are compatible with Figs. 2.1 and 2.2, we think each of these species needs much more study to elucidate its unique traits. Also we have been studying the specific roles of invertases in plant tissue types (Chen 1992; Chen and Black 1992) and during this work we produced antibodies to soluble acid invertase from soybeans. Using that antibody, at near equal amounts of soluble leaf protein on each gel, we were unable to convincingly detect acid invertase in leaf extracts from polysaccharide-utilizing CAM plants. In contrast, the hexose-utilizing CAM plants stained positively in the same study (Fig. 2.3). Other interpretations, such as

Table 2.3. Taxonomic distribution of some enzymes of carbohydrate metabolism in CAM plants

Family, species	ADPG-PPase <sup>a</sup>	PP <sub>i</sub> -PFK	ATP-PFK	ATP-FK	ATP-GK	AI	NI	SPS	PEPCK	NADP-ME	NAD-ME	PPDK
Asclepiadaceae, <i>Hoya carnosa</i>	30	624-872	2-26	6	7	27-45	6-9	32	610	207	52	12
Bromeliaceae, <i>Ananas comosus</i>	9	1772-2714	33-83	3	11	203-495	11-20	3	2160	142	18	6
Clusiaceae, <i>Clusia rosea</i>	107	20-29	53-127	174	ND <sup>b</sup>	42-262	16(3)	27	1260	ND	54	111
Asphodelaceae, 3 <sup>c</sup>	4	15(5)	109(5)	10	6	17(5)	11(4)	40	1050	64	48	108
Crassulaceae, 5 <sup>d</sup>	62(3)	31(6)	302(7)	4-13	2-35	97(5)	15(7)	31(3)	153(3)	178(3)	195(3)	124(3)

<sup>a</sup>ADPG-PPase: ADP glucose pyrophosphorylase

PP<sub>i</sub>-PFK: pyrophosphate-dependent phosphofructokinase (also known as PFP, or pyrophosphate : fructose 6-phosphate 1-phosphotransferase)

ATP-PFK: ATP-dependent phosphofructokinase

ATP-FK: ATP-dependent fructokinase

ATP-GK: ATP-dependent glucokinase

All activities are in nmol min<sup>-1</sup> mg<sup>-1</sup> protein. All values followed by ( ) are means for the number of samples in parentheses

Assay temperature was 37°C for SPS, 25°C for others

<sup>b</sup>Not detectable

<sup>c</sup>*Aloe arborescens*, *A. ciliaris*, *A. vera*.

<sup>d</sup>*Sedum telephium*, *Crassula rupestris*, *C. argentea*, *Kalanchoë fedtschenkoi*, *K. daigremontiana*.

AI or NI: acid or neutral invertase

SPS: sucrose 6-phosphate synthase

PEPCK: PEP carboxykinase

NADP-, NAD-ME: NAD(P)-dependent malic enzyme

PPDK: pyruvate, phosphate dikinase.

poor antibody cross reactivity between plants, or the possible presence of competing protein inhibitors are possible (Chen 1992). We interpret these results as indicating that less acid invertase or a different antigenic protein is expressed in CAM plants that utilize polysaccharides. We emphasize that acid invertase activity is detectable in both PEPCK- and ME-CAM plants (Table 2.3), but the hexose-utilizing PEPCK-CAM plants show higher activities. This result supports the metabolic sequence for near equal hexose synthesis as depicted in Fig. 2.2 and measured in pineapple and *Clusia* (Table 2.2).

## 2.5 Bioenergetics in Different Groups of CAM Plants

To calculate the stoichiometries between acidity and malic acid we assume  $2 \text{ H}^+$ /malic acid and that each hexose is equivalent to 2 PEP molecules, hence 2 malic acid molecules. We have not calculated the energetics of all metabolites crossing membranes, e.g. a hexose-P or triose-P crossing the plastid envelopes; but we assume 1 ATP is used per  $2 \text{ H}^+$  and 1 malic acid (Lüttge et al. 1981) transferred into the vacuole. Note that the exact biochemical route of starch breakdown in the chloroplast is not known. Thus, in CAM plants that use polysaccharides the net cost of nocturnal conversion and storage is 1 or 2 ATPs for 2 malic acids, depending on whether starch breakdown is phosphorolytic (Paul et al. 1993) or amylolytic, respectively (Fig. 2.1). The next day 13 ATPs are needed to convert 2 malic acids to a hexose equivalent stored as starch. The energy costs are similar in hexose-storing plants (Fig. 2.2), with 2 ATPs (or ATP equivalents) required per 2 malic acids at night and 10.5 ATPs (or ATP equivalents) per hexose synthesized the next day. These calculations include the PCR cycle.

These CAM cycles, as given in Figs. 2.1 and 2.2, are deceptively simple and straightforward. In fact, they have many unknown aspects; e.g. little information is available on the formation or potential utilization of  $\text{PP}_i$  as an energy source, either at the interconversion of F 6-P and F 1,6- $\text{P}_2$  or from the synthesis of sucrose or a polysaccharide. The movements of the substrates and energy sources into various cellular compartments is poorly understood, e.g. putatively the hexokinases needed in Fig. 2.2 are in mitochondria and control mechanisms for many of the enzymes are poorly studied.

The theory we propose for the overall daily carbon flow in both Fig. 2.1 and Fig. 2.2 is that there is a large carbon flow at night pulled, via a  $\beta$ -carboxylation of PEP, toward malic acid synthesis, by sequestering malic acid in the vacuole with a low overall energy cost (either 0.5 or 1 ATP, depending on the pathway of carbohydrate breakdown). Then, the next day, energy derived from photosynthesis would drive the reverse synthesis of carbohydrates. During the day the extra energy input, 2.25 to 3.5 ATP per malate, would mainly arise from light energy, whereas the lower energy requirement at night would be met by dark respiration (Figs. 2.1 and 2.2). The fixation of  $\text{CO}_2$  in both instances would then have the additional energy requirements of  $3 \text{ ATP} : 2 \text{ NADPH} : 1 \text{ CO}_2$ .

## 2.6 Conclusions

In green CAM cells there is a massive biochemical commitment of between 8 and 20% of the total cell dry matter into a daily cycle. This diel cycle, in its simplest context, supplies much of the  $\text{CO}_2$  for photosynthesis.

CAM plants can be divided into two large groups based upon the primary carbohydrate reservoir used in their daily cycle. Some CAM plants utilize polysaccharides as their carbohydrate reservoir, while others utilize soluble hexose sugars. These two groups of CAM plants mostly decarboxylate malate either with a malic enzyme or with PEP carboxykinase, respectively. Each group of CAM plants also expresses other characteristic sets of enzymes. Hence the sequence of metabolic reactions vary markedly between plants that store polysaccharides versus the storage of hexoses each day. The subcellular site of carbohydrate storage also varies, polysaccharides being stored in chloroplasts and soluble hexoses in the vacuole.

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